Developments in new TB biomarkers

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- Why do we need biomarkers so badly?
- Biomarkers of vaccine immunogenicity
- Biomarkers for (early) diagnosis
- Correlates of protection
- New developments in TB biomarkers
- Biomarkers in TBVAC2020
Global pipeline of TB vaccine candidates

Foundation to facilitate European efforts towards the global development of new TB vaccines

www.tbvi.eu
How can we select better candidates?

*shifting the risk curve...*
Biomarkers of immunogenicity following vaccination
Immunogenicity of BCG in UK infants: IFNg

- 3 months after BCG vaccination at 3-13 weeks of age, 100% of the UK infants given BCG vaccination made IFN\(\gamma\) responses >62pg/ml to PPD; none of the unvaccinated infants were IFN\(\gamma\) responders (Lalor et al JID 2009, 199:795)
TBVAC-associated sub-unit TB vaccines: MVA85A

Phase 1 – trial in PPD neg

MVA85A alone or BCG + MVA85A

Immunogenicity: excellent primary response

Safety: low reactogenicity

Immunogenicity in MVA-85A vaccine trial: Ag85A-specific T cell responses were induced (IFN-γ ELISpot, 7 days post-MVA85A boost)

Tameris M et al, Lancet 2013
Biomarkers of infection

- Better tools to confirm TB diagnosis
Detection of Tuberculosis in HIV-Infected and Uninfected African Adults Using Whole Blood RNA Expression Signatures: A Case-Control Study

Myrsini Kaforou¹,²,³, Victoria J. Wright¹,²,², Tolu Oni¹,³,⁴, Neil French⁴,²,⁵,⁶, Suzanne T. Anderson⁷,⁸, Nonzwakazi Bangani³, Claire M. Banwell⁷,⁸, Andrew J. Brent¹,⁹, Amelia C. Crampin⁴,⁶, Hazel M. Dockrell¹⁰, Brian Eley¹¹, Robert S. Heyderman⁸,¹², Martin L. Hibberd¹³, Florian Kern⁷, Paul R. Langford¹, Ling Ling¹³, Marc Mendelson¹⁴, Tom H. Ottenhoff¹⁵, Femia Zgambo⁴, Robert J. Wilkinson¹,³,¹⁶, Lachlan J. Coin²,¹⁷, Michael Levin¹,⁴

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• Patients with confirmed culture positive TB compared to those with LTBI and with Other Diseases (pneumonia, malignancy, meningitis etc); HIV+ and HIV-ve

• PaxGene tubes, Illumina microarrays, Elastic Net

• Clustering based on disease state, not geographical location (Malawi or South Africa) or HIV status

• Signature was developed based on all samples, irrespective of site or HIV status.

Kaforou et al, Plos Med 2013: 10:e1001538
Gene expression signatures could distinguish TB from LTBI or from other diseases (OD)

Training 80%
Test 20%
TB vs LTBI, n= 285, 76
TB vs OD, n= 293, 76

Red: up reg, green down reg;
Purple TB, green LTBI, light blue OD
Signatures work in both HIV+ and HIV-ve subjects…
Biomarkers of progression from latent to active tuberculosis

- Early diagnosis would reduce transmission of TB
WP 3: Natural protective immunity against TB
HIV -ve newly diagnosed Pulmonary TB patients
Household contacts

WP 4: Impact of HIV-1/AIDS and response to treatment on immunity against TB
HIV +ve individuals

Diagram:
- Exposure to TB
- 6 months
- 18 months
- 2 years
- HIV infection
- Prophylactic treatment of LTBI
- ART treatment
- Protected
- Not protected

Grand Challenges in Global Health #6-74
14 HIV+ progressors and 15 non progressors tested using dcRT-MLPA for 141 genes

IL-13 and AIRE (APECED, autoimmune regulator) were able to give good prediction of TB development up to 8 months later

Type 1 interferon signalling signatures in those expressing IL-13, suggestive of some pathology?
What about biosignatures of protective immunity?
We started with the obvious candidates...
SATVI Project: To identify BCG-induced immune correlates of protection against TB

BCG

Blood collected, processed, stored

n=5,675

BIRTH

10 weeks

2 years

Identify infants who are:

Protected against TB

n=91

Not Protected against TB

n=45

Compare specific immunity at 10 weeks at age

PI: Willem Hanekom
No difference in frequency of BCG-specific polyfunctional T cells between protected and unprotected infants

From 5,724 enrolled infants:
- TB cases (n=29)
- Community controls (n=55)
- Household controls (n=55)

IL-2
IL-17
IFN-γ
TNF

Kagina B. et al. AJRCCM 2010;
BCG given at birth. Infants followed for 2 years to assess protection.
Whole blood incubation with BCG for 12 hrs, at 10 weeks of age, intracellular cytokine staining.
Biomarkers indicating protective immunity: gene expression profiles

- Induced by a protective vaccine (not yet...)
- Gene signatures that normalise after successful drug treatment
- In those who do not relapse after treatment (compared to those who relapse although apparently successfully cured) (also in those with TB and diabetes who are harder to treat successfully)
- In those who remain healthy with long-standing latent infection
Gene expression profiles show normalisation after TB treatment and are helping identify new biomarkers and overlooked importance of innate immunity.
Biomarkers indicating ability to inhibit growth of live mycobacteria
Mycobacterial growth inhibition as an unbiased measure of TB vaccine-induced immunity

Straight to MGIT reference tube

Sample A

Sample B

Incubate diluted blood and mycobacteria for 4 days

Lyse cells, extract remaining mycobacteria and add to MGIT tube

Time to positivity (TTP) in days and hours
New Human Challenge Model – quantifying BCG at vaccine site

- BCG given id to volunteers and punch biopsy performed
- BCG quantified by PCR (Fig B) or culture
- Cells isolated from suction blisters were mainly CD15+ neutrophils
- Re-vaccinated subjects showed lower BCG genome copies (Fig C)

Minassian et al JID 2012 205: 1035
Developing biomarkers of protective immunity

– Very hard as we don’t understand tuberculosis
– Currently, we are closer to developing biomarkers for infection than of protection
– Hopefully we can learn from ongoing vaccine trials
– Measuring what really matters - mycobacterial growth inhibition or human challenge assays - may help identify useful biomarkers
– Explore new developments such as “memory” or imprinting in innate immunity, epigenetics of memory T cells, systems biology
Final thoughts...the way forward

- We are getting closer to a much more objective view of the immunology of tuberculosis, guided by the results from gene expression analysis using microarrays and RNAseq.

- Meanwhile technology is developing too...moving beyond lateral flow tests to exploit nanotechnology etc.

- Biomarkers of protection need more effort but we will hopefully learn from the ongoing studies....

- We really do need better biomarkers to accelerate TB vaccine development; we hope the coordinated efforts through TBVI/TBVAC2020 will help...
Stage Discovery: Bottom up

Discovery of novel TB vaccine strategies

Development of clinically relevant animal models

Preclinical of selected candidates: Portfolio management (top down)

Early clinical selected candidates: Portfolio management (top down)

Correlates of protection

Discovery

Gate 1

Preclinical

Gate 2

Ph 1-2a

Gate 3
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